Research Article | Vol. 14, No. 6 / 1 Jun 2023 / Biomedical Optics Express 2920
Biomedical Optics EXPRESS

Identification of late-life depression and mild cognitive impairment via serum surface-enhanced Raman spectroscopy and multivariate statistical analysis

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Abstract: Identification of age-related neuropsychiatric disorders, i.e., late-life depression (LDD) and mild cognitive impairment (MCI) is of imperative clinical value considering the large probability of misdiagnosis and current lack of sensitive, non-invasive and low-cost diagnostic approaches. Here, the serum surface-enhanced Raman spectroscopy (SERS) technique is proposed to identify healthy controls, LDD and MCI patients. Based on SERS peaks analysis, abnormal levels of ascorbic acid, saccharide, cell-free DNA and amino acids in serum are found to be potential biomarkers for identifying LDD and MCI. These biomarkers might be related to oxidative stress, nutritional status, lipid peroxidation and metabolic abnormalities. Moreover, partial least square analysis-linear discriminant analysis (PLS-LDA) is applied to those collected SERS spectra. Finally, the overall identification accuracy is 83.2%, and accuracies are 91.6% and 85.7% for differentiating healthy versus neuropsychiatric disorders and LDD versus MCI, respectively. Thus, the serum SERS combined with multivariate statistical analysis has proved its successful potential for rapid, sensitive and non-invasive identification of healthy, LDD and MCI, which may open new avenues for early diagnosis and timely intervention for age-related neuropsychiatric disorders.

1. Introduction

Currently, age-related neuropsychiatric disorders greatly increase with the extended lifespan and improved public health. Among them, late-life depression (LDD) and Alzheimer's disease (AD) are the most popular in the elderly. LDD is commonly charactered with depressed mood, sleep disorders, negative emotions and/or morbid thoughts [1]. Almost 20% of people over 65 have depressive symptoms, while the percent reaches 40% in people above the age of 85 [2]. Generally, LDD is controllable and curable if timely diagnosis and appropriate treatment are performed. However, the onset of LDD is often ignored or covered up by aging or aging-related diseases resulting less than 20% of them are timely diagnosed and aggressively treated [2]. AD,

#487939 https://doi.org/10.1364/BOE.487939
Journal © 2023 Received 16 Feb 2023; revised 16 Apr 2023; accepted 9 May 2023; published 25 May 2023

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as one popular aging-related disease, is a severe progressive, irreversible neurodegenerative disorder of the brain characterized with accumulation of amyloid plaques and neurofibrillary tangles, causing memory loss and cognition decline. The incidence of AD is nearly 5–8% in people over 65, while the percent increases to 25–50% as the age rises over 85 [3]. In general, AD progresses along a continuum from preclinical disease, to mild cognitive impairment (MCI) and then AD dementia with a median survival time of 5 years after diagnosis [3,4]. So far, there is no effective therapies for AD, early recognition and intervention of MCI has been demonstrated particularly significant to slow disease progression and increase the survival of patients [4,5]. However, LLD and MCI have similar clinical presentations [6,7], such as anxiety, loss of memory and cognitive impairment, as well as share a number of pathophysiology processes, including neurodegenerative changes, alterations to cerebrovascular functioning and high inflammatory cytokine levels, resulting in difficulty to correctly identify. Thus, early and accurate diagnosis of LLD and MCI is necessary for timely decision on precise intervention and therapy.

Neuropsychiatric disorders in clinical are normally diagnosed by several methods include neuropsychiatric questionnaires [8], biomedical imaging [9] and biomarkers analysis in cerebral spinal fluid (CSF) and blood [10,11]. Neuropsychiatric questionnaires (such as Mini-Mental State Examination (MMSE), Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and Montreal Cognitive Assessment (MoCA)) are the most frequently used but are subjective and unable to predict the onset of disease [8]. Biomedical imaging of brain (such as computerized tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET)) is characterized with high diagnostic accuracy, but suffers from time and cost-consuming [9]. Biomarkers (such as β-amyloid (Aβ), phosphorylated tau 181 (P-tau181) and t-tau (T-tau) protein) detection in CSF is less cost but is too invasive (lumbar puncture) and always needs special equipment for sample analysis [10]. Although blood biomarkers, such as T-tau, P-tau and Aβ42 have been studied in recent years and show excellent performance in detection of neuropsychiatric disorders, but various sensitivity, specificity and high false positive rate limit their clinical applications [11]. Hence, developing a rapid, sensitive and non-invasive diagnostic method would be of significant clinical value to identify LDD and MCI for timely intervention and rationally therapy for the individual patient.

As previous studies have indicated close associations between neuropsychiatric disorders and metabolism, including mitochondrial energy metabolism [12], glucose metabolism [13] and amino acid metabolism [14], metabolic variations detection might be a substitute for traditional methods in identification of LDD and MCI patients. Coincidentally, Raman spectroscopy (RS), as a fast, non-destructive and label-free spectroscopic technique, can provide conformational structure and specific information about metabolic variations by measuring the inelastic scattering between monochromatic photons and detected molecules [15,16]. Peripheral blood, is commonly used as the most ideal specimen for Raman acquisition, because it typically contains rich biochemical information of molecules from metabolic changes and body microenvironment as well as can be collected and measured with high convenience and repeatability. Although the spontaneous Raman signal of blood sample is relatively weak, by the presence of a plasmonic structure in the close vicinity of the target analyte, i.e., surface-enhanced Raman spectroscopy (SERS) [17], the Raman signal can be amplified by 10^{14} times based on electromagnetic enhancement (EME) and chemical enhancement (CE) [18]. With the characteristics of flexibility, non-invasive and sensitivity, RS and SERS have attracted a growing attention and are frequently used in disease diagnosis, including diagnosis of neuropsychiatric disorders. Carota *et al.* measured the blood serum Raman spectra from healthy and AD patients, and lower level of carotenoids were detected in blood from AD patients. Good discrimination with correct predictions of 93% demonstrated the possibility to use Raman spectroscopy for the diagnosis of AD [19]. Paraskevaidi *et al.* collected Raman spectra of blood plasma from early-stage AD, late-stage AD, dementia with *Lewy bodies* and healthy controls. High classification accuracy over 80% for the different groups

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were achieved, and important biomolecules such as amide II, lipid and phenylalanine can serve as a panel of biomarkers for diagnosis of neurodegeneration disorders [20]. Although these results suggest Raman spectroscopy is a sensitive, cost-effective and blood-based method for diagnosis of neurodegeneration disorders, to the best of our knowledge, characterization of blood samples from LDD and MCI patients by RS or SERS has not yet been studied. Thus, fast and accurate detecting and analyzing of metabolic changes in blood samples between LDD and MCI patients is greatly significant for early diagnosis and rational intervention.

In this paper, the SERS spectra are collected from blood serum mixed with Au@Ag nanoparticles aggregates (Au@AgNAs) colloidal substrate to explore the metabolic differences among healthy control, LDD and MCI patients. Significant variations in ascorbic acid, saccharide, cell-free DNA and amino acids levels are observed in SERS peaks analysis. After PLS-LDA-based multivariate statistical analysis, the overall accuracy is 83.2% for identifying healthy, LDD and MCI patients. Hence, the serum SERS technique combined with PLS-LDA has proved its successful potential for rapid, sensitive and non-invasive identifying healthy, LDD and MCI, which can open new avenues for early diagnosis and timely intervention for neuropsychiatric disorders.

2. Materials and methods

2.1. Sample preparation and SERS measurements

In this paper, 40 healthy controls (70.4 ± 5.2 years, 19 male and 21 female) and 79 neuropsychiatric disorders, i.e., 43 LDD cases (70.8 ± 8.3 years, 8 male and 35 female) and 36 MCI cases (74.2 ± 8.7 years, 12 male and 24 female) were carefully chosen from the Ningbo Kangning Hospital in China based on the clinical assessments, biomedical imaging and neuropsychological tests. This study was conducted in accordance with the Declaration of Helsinki. An ethical approval (No. NBKNYY-2021-LC-56, Dec./31/2021) has been obtained and written informed consent was signed by each above patient. 3 ml overnight fasting peripheral blood was taken from each subject between 7:00 to 8:00 A.M. Each blood sample was centrifuged at 3000 rpm for 10 min at 4°C to collect the supernatant. These serum samples were rapidly stored at −80°C freezer.

For SERS measurements, a substrate of core-shell Au@AgNAs colloidal solution was made by Ag deposition on the surface of Au using the seed-growth method [21]. Firstly, AuNPs (diameter is about 32 nm) were synthesized by the reduction of $HAuCl_4 \cdot 4H_2O$ using trisodium citrate [22]. Then, the above AuNPs seeds (3 ml) were sonicated for 10 min and mixed with ascorbic acid solution (150 µl,10 mM). Afterwards, $AgNO_3$ solution (150 µl,10 mM) was dropped into the above solution with shaking at 1000 rpm. Continuous shaking for 7 min was done after the color of the solution became orange. Finally, the core-shell Au@AgNAs colloids was synthesized.

4 ml above colloidal solution was centrifuged at 10,000 rpm for 10 min to achieve the final concentration by discarding 3.8 ml supernatant. Then, the thawed serum was mixed with above Au@AgNAs in 1:1 proportion and incubated for 2 h at room temperature. 2µl of the mixture was dropped onto an aluminum substrate and air dried for SERS measurements. The SERS spectra were obtained by using a Renishaw inVia Qontor confocal Raman spectrometer (Renishaw, UK) coupled to a Leica microscope with a 50× objective (NA = 0.50) in the backscattering geometry. SERS signal was excited by a 785 nm laser and measured in a wavenumber range from 400 cm^{-1} to 1800cm^{-1} with a spectral resolution of 1 cm^{-1}. In order to reduce the operation variations and coffee-ring effect, repeated measurements were collected at five different positions (including the middle and the edge) and the mean of these five measurements served as the final SERS spectrum of each subject for further processing and analysis. The detailed workflow of the above process was demonstrated in Fig. 1.

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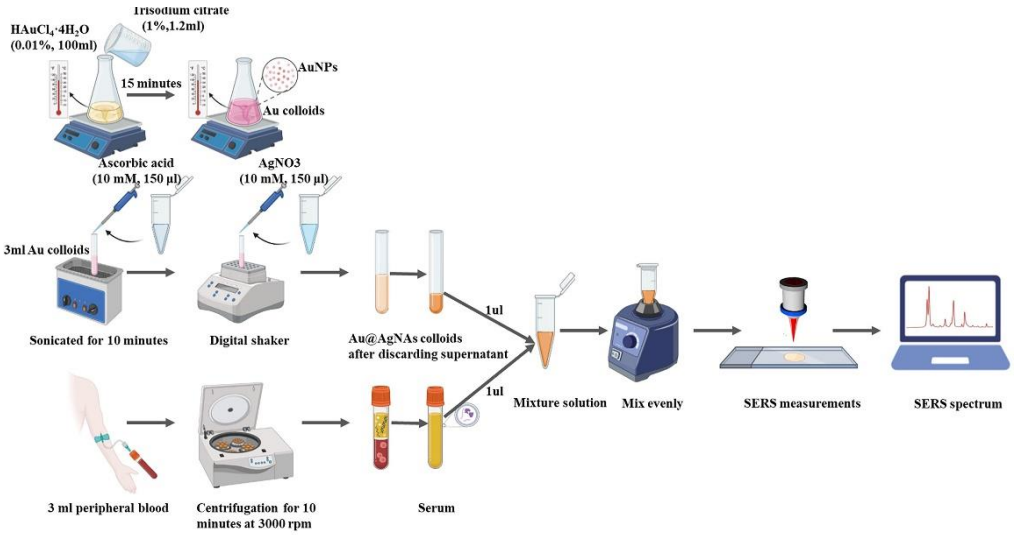

Fig. 1. The workflow of the sample preparation and SERS measurement.

2.2. Spectral preprocessing and analysis

SERS spectra were successively preprocessed by Savitzky-Golay-based smooth [23], the fifth order polynomial fitting-based fluorescence background removal and maximum normalization [23,24].

After the above preprocessing, the assignments of Raman-active vibrational modes and biochemical components were performed on the serum SERS peaks. Two-sample t-test was then applied on these SERS peaks' intensities between the healthy controls versus neuropsychiatric disorders and the LDD versus the MCI, respectively, and only the biochemical components with statistically significant differences ($p < 0.05$) were treated as the potential diagnostic biomarkers. Finally, multivariate statistical analysis of PLS-LDA was employed to establish classification models. And specifically, a two-step classification scheme was carried out by two binary classifiers; i.e., a binary classifier was first used to differentiate healthy versus neuropsychiatric disorders groups and then a second binary classifier was further used to differentiate LDD versus MCI groups. In the above models, PLS analysis was first conducted on the SERS spectra and the first 15 PLS scores were extracted, then, only the PLS scores with statistically significant difference selected by two-sample t-test were put into subsequent LDA to train each classification model. Both models were evaluated by leave-one-out (LOO) cross-validation, in which the classification accuracy, sensitivity, specificity, receiver operating characteristic (ROC) curves and the area under ROC (AUC) were used as the criteria to evaluate their performances.

3. Results and discussions

3.1. Characterization of the Au@AgNAs colloids

For SERS measurements, silver colloids (AgNPs) and gold colloids (AuNPs) are the most frequently used SERS substrates [25]. Generally, AuNPs synthesis is simple and controllable, but the enhancement ability is weak. In contrast, much stronger enhancement factor can be generated by AgNPs, while nonuniform shapes result in poor repeatability of SERS signal.

In this study, Au@AgNAs colloids is created and used as SERS substrate. The characterizations by transmission electron microscopy (TEM), UV/visible absorption spectroscopy and the corresponding size distribution are plotted in Fig. 2(a), which shows the almost uniform core-shell

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structure with a mean diameter of approximately 50 nm including the outer Ag shell thickness of 8.2 nm, and two peaks at 400 nm and 500 nm corresponding to the plasmon resonance of Ag shell and Au core, respectively. Furthermore, the Raman enhancement ability and reproducibility of Au@AgNAs colloids are evaluated using 4-MBA as the probe molecule, as shown in Fig. 2(b) and Fig. 2(c). By comparison the spectra of spontaneous Raman spectrum and SERS spectrum of 4-MBA absorbed on the different colloids in Fig. 2(b), the highest enhancement effect is observed in sample of 4-MBA on the synthesized core-shell Au@AgNAs. Moreover, 150 SERS spectra are obtained within a selected area by mapping for 4-MBA on core-shell Au@AgNAs, the consistent spectral shape in Fig. 2(c) and uniform SERS signal with slight fluctuation from different spectra at peak of 1078 cm^{-1} in Fig. 2(d) are intuitively evidenced by the consistent distribution of these 150 intensities with a calculated RSD (relative standard deviation, RSD) value of only 3.6%. These results are consistent with those in published papers and demonstrated that core-shell Au@AgNAs is an effective SERS substrate with characteristics of size-uniformity, strong enhancement ability and fine reproducibility.

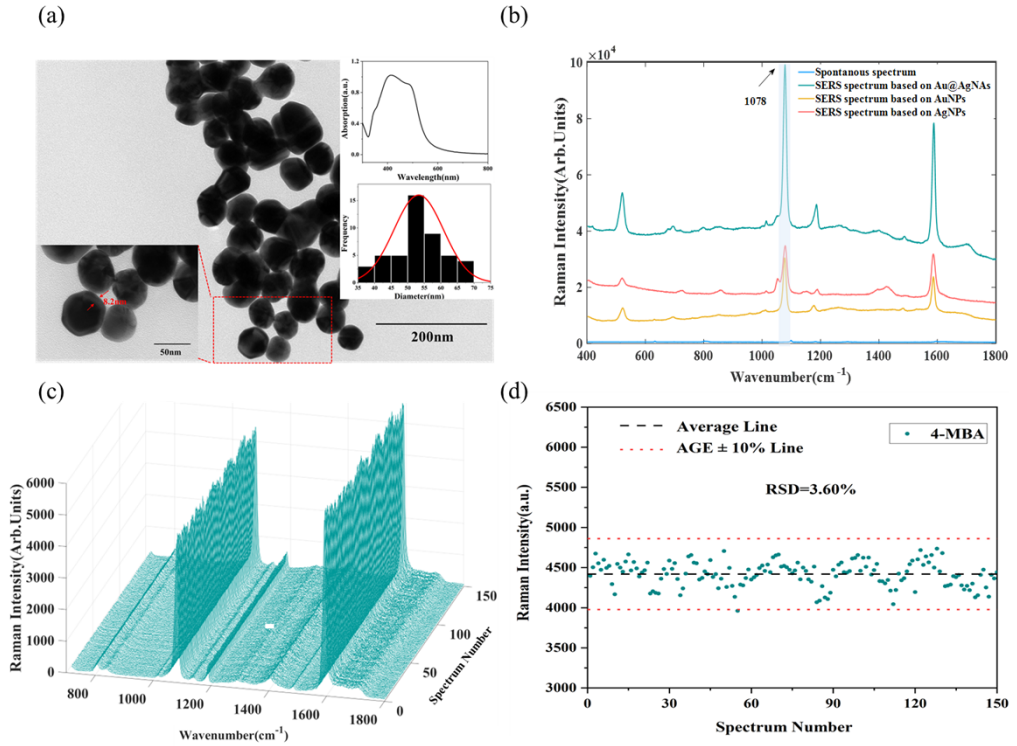

Fig. 2. (a) TEM images, UV/visible absorption spectrum and size distribution of core-shell Au@AgNAs; (b) Comparison of spontaneous Raman spectrum of 4-MBA and SERS spectrum of 4-MBA absorbed on the prepared core-shell Au@AgNAs, AuNPs (50 nm) and AgNPs (50 nm), respectively; (c) 150 SERS spectra obtained within the selected area by mapping for 4-MBA on core-shell Au@AgNAs; (d) Distribution of SERS intensities at peak of 1078 cm^{-1} from the total 150 SERS spectra.

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3.2. SERS peaks analysis among different groups

3.2.1. SERS peaks' tentative assignments

After preprocessing, the mean SERS spectra of healthy, LDD and MCI groups are plotted in Fig. 3. By visual inspection, the primary SERS peaks can be found at 493, 592, 638, 725, 813, 887, 1003, 1134, 1207, 1331 and 1656 cm^{-1}. The detailed vibrational modes and biochemical components assignments of these peaks are listed in Table 1 according to the previously published studies [19,20,26,27].

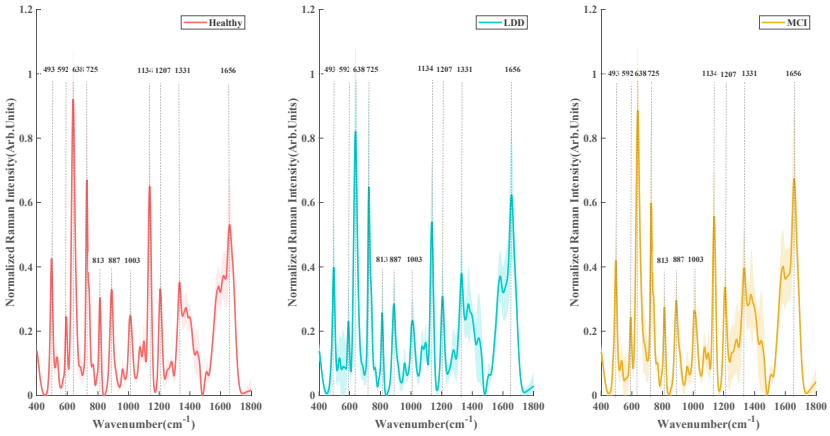

Fig. 3. The average serum SERS spectra after preprocessing from the healthy, LDD and MCI groups, in which the shaded areas represent the standard deviations among each group.

Table 1. Tentative assignments of the primary SERS peaks of blood serum [19,20,26,27].

Peak position (cm^{-1})	Assignments
493	Ring vibration, Cellulose, guanine, L-Arginine
592	Ascorbic acid, amide-VI
638	C-S stretching vibration, L-Tyrosine, lactose
725	C-H bending vibration, Adenine, coenzyme A
813	C-C-O stretching vibration, L-Serine, glutathione
887	C-O-H bending vibration, Glutathione, D-(+)-galactosamine
1003	C-C symmetric stretch, Phenylalanine
1134	C-N stretching vibration, D-Mannose
1207	Ring vibration, L-Tryptophan, phenylalanine
1331	C-H stretching vibration, Nucleic acid bases, D-mannose
1656	C = O stretching vibration, Amide-I, α-Helix

3.2.2. Healthy versus neuropsychiatric disorders

Figure 4(a) and (b) show the average serum SERS spectra of healthy and neuropsychiatric disorders groups as well as the subtracted spectrum, respectively. After performing two-sample t-test on intensities of the primary SERS peaks in Table 1, five SERS peaks at 813 cm^{-1} ($p = 0.0010$), 887 cm^{-1} ($p = 0.0056$), 1134 cm^{-1} ($p = 0.0032$), 1331 cm^{-1} ($p = 0.0353$) and 1656 cm^{-1} ($p = 0.0017$) are discovered to be with statistically significant differences between the

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healthy and neuropsychiatric disorders groups, as shown in Fig. 4(c). Specifically, SERS intensity at 813 cm^{-1} is lower in neuropsychiatric disorders group compared to that in healthy group, which means less L-serine in the serum of neuropsychiatric disorders patients. L-serine is a polar amino acid and its residues serve as a primary site for phosphorylation, phosphorylation status is closely associated with the ability to alter protein interactions and conformation, which is critical for cellular metabolism and neurological function [28]. Dysfunctional phosphorylation, particularly of tau protein, is found in neuropsychiatric disorders patients [29,30], which might result in a decreasing concentration of L-serine in blood serum. A similar trend of decreasing SERS intensities at 887 and 1134 cm^{-1} corresponding to lower glutathione and saccharides are also found in neuropsychiatric disorders group. Glutathione is a major antioxidant that can eliminate free radicals (such as reactive oxygen species) and play an important role in regulating redox balance and oxidative stress [31]. However, excess oxidative stress in brain disorders impairs the protein expression and activity of glutathione-related enzymes (such as GPx, GR, GST, and GCL), resulting in its abnormal synthesis and metabolism [32]. In addition, glutathione synthesis is also related to mitochondrial functions [32]. The impaired mitochondrial energy metabolism (such as NAD^+, NADH, ATP, pyruvate, and lactate) result from oxidative stress might reduce the production of NADPH [33], which is a coenzyme that mediates glutathione synthesis, thus finally lead to less glutathione in neuropsychiatric disorders group. Coincidentally, many previous studies conducted in animal models and clinical patients with brain disorders have demonstrated decreased levels of glutathione in peripheral blood of these individuals [31,32,34]. Furthermore, glutathione deficiency often occurs prior to neuropathological abnormalities [32] and might can be used as a biomarker for early diagnosis of neuropsychiatric disorders. In brain, saccharides are normally glycoconjugated to proteins or lipids to help structural development, synaptogenesis and synaptic transmission [35]. The decreasing saccharides in blood of neuropsychiatric disorders might be due to the poor neurological functioning from abnormal glycoprotein and ganglioside metabolism in brain disorders.

In contrast, SERS peak at 1331 cm^{-1} show higher intensity in neuropsychiatric disorders patients compared with it in healthy control, which suggests more cell-free DNA in blood from neuropsychiatric disorders patients. Normally, circulating cell-free DNA is a product of cell death. Once cell death, it shrinks and degrades its plasma membrane, and ultimately rupture and release its contents into the blood, including DNA fragments [36]. Thus, the increasing of circulating cell-free DNA might result from the excess cell death in brain derived from the oxidative stress in neuropsychiatric disorders. The larger SERS peak of 1656 cm^{-1} in the neuropsychiatric disorders group indicates more free amino acids in blood serum, which is in agreement with findings in several recent studies [37–39]. Amino acids play essential roles in the control of brain functions by acting as regulators of the energy metabolism [37]. Alterations in blood free amino acid might be influenced by the compromised energy metabolism including nitrogen metabolism [38] and cerebral glucose metabolism [40] in neuropsychiatric disorders patients.

The above results demonstrate that obvious variations of the biomolecules, such as L-serine, glutathione and saccharides among different groups, suggesting promising potential for identifying neuropsychiatric disorders using serum SERS technique.

3.2.3. LDD versus MCI

Figure 5(a) and (b) show the average serum SERS spectra of LDD and MCI groups as well as the subtracted spectrum, respectively. After performing two-sample t-test on intensities of the obvious SERS peaks, four SERS peaks at 592 cm^{-1} ($p = 0.0245$), 1134 cm^{-1} ($p = 0.0042$), 1331 cm^{-1} ($p = 0.0287$) and 1656 cm^{-1} ($p = 0.0025$) are identified to be with statistically significant differences between LDD and MCI groups, as shown in Fig. 5(c). In particular, SERS intensity at 592 cm^{-1} is lower in MCI group than that in LDD group, demonstrate less ascorbic acid is in MCI group. Many studies indicate that low level of ascorbic acid is associated with depression [41,42]

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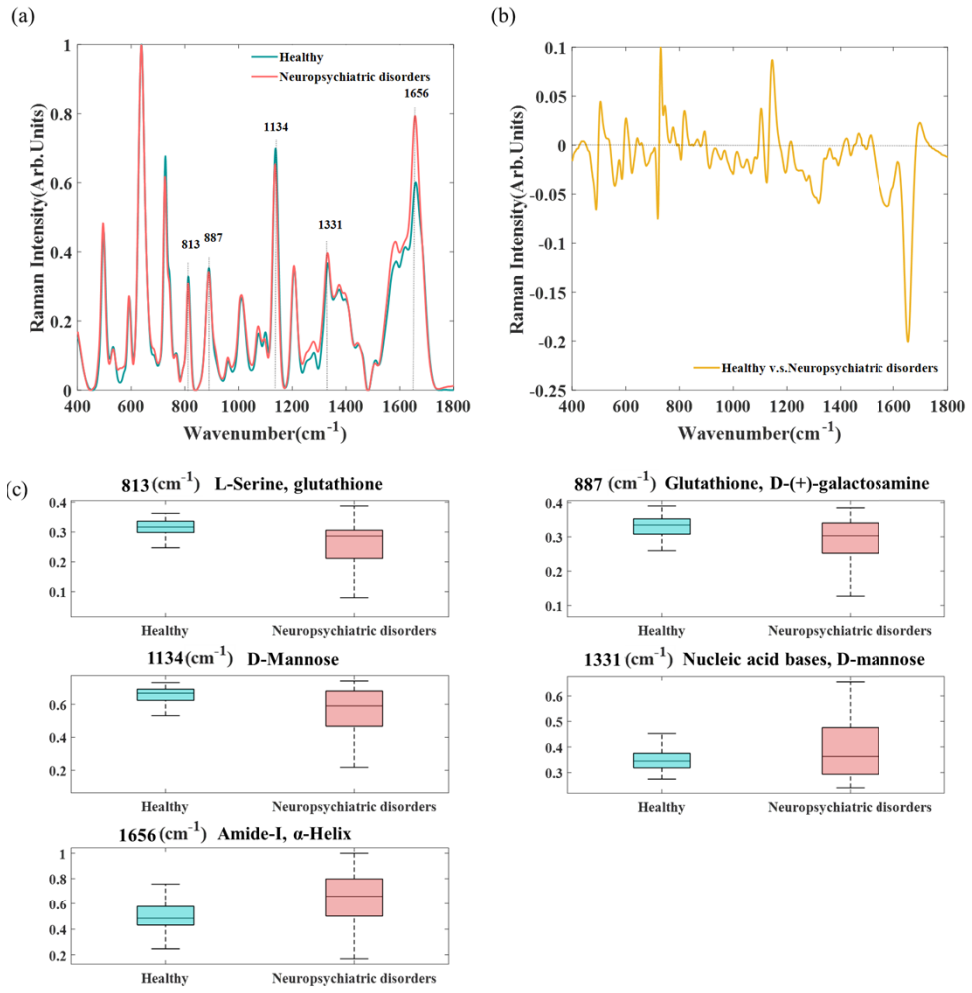

Fig. 4. **(a)**The comparison of average serum SERS spectra between healthy and neuropsychiatric disorders groups; **(b)** the subtracted spectrum between these two groups; **(c)** the boxplots of SERS intensities with statistically significant differences between these two groups at peaks of 813, 887, 1134, 1331 and 1656 cm^{-1}.

and cognitive impairment [43,44], and lower ascorbic acid status is linked to greater cognitive impairment. Ascorbic acid is a remarkable potent water-soluble antioxidant without synthesis in the brain, which catalyzes the reduction of superoxide radicals and plays a crucial role in maintaining oxidative balance [42]. Increased consumption of ascorbic acid by the oxidative stress in brain might lead to its lower levels in serum of MCI patients [43,45]. The lower SERS intensity at 1134 cm^{-1} corresponding to D-mannose suggests less saccharide in blood serum of MCI group. In brain, saccharides are commonly conjugated to proteins or lipids to help structural development, synaptogenesis and synaptic transmission [35], which have beneficial effects on neuronal and cognitive function. Many studies demonstrated a high concentration of Aβ deposits in MCI and AD patients [46–48], in which Aβ are produced by a glycoprotein called amyloid precursor protein [49], this might result in a decreasing level of saccharide in blood of MCI patients. Another possible reason might be the poor nutritional status in the brain of MCI patients lead to the abnormal saccharide metabolism [35].

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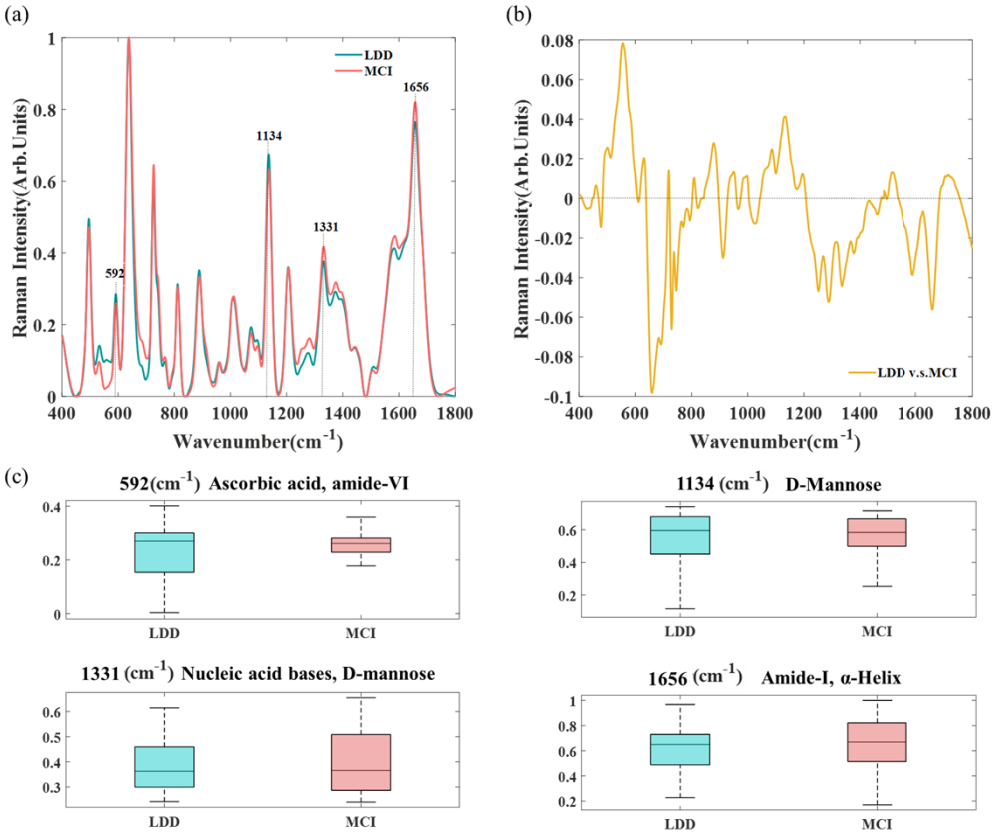

Fig. 5. **(a)** The comparison of average serum SERS spectra between LDD and MCI groups; **(b)** the subtracted spectrum between these two groups; **(c)** the boxplots of SERS intensities with statistically significant differences between these two groups at peaks of 592,1134, 1331 and 1656 cm^{-1}.

On the contrary, higher intensities at 1331 and 1656 cm^{-1} indicate more free nucleic acid bases and amino acids in blood serum of MCI group than that in LDD group. Increased cell-free DNA could be attributed to the fact that more cell death result from intensive oxidative stress in MCI patients [36,50]. In addition, severe DNA damaging from aldehydes produced by increased lipid peroxidation in MCI might also lead to an increasing level of DNA fragments in blood [51].

These findings suggest that the levels of ascorbic acid, saccharide, cell-free DNA and amino acids in serum are expected to be promising indicators for identifying LDD and MCI patients.

3.3. Multivariate statistical analysis of SERS spectra among different groups

In multivariate statistical analysis, the PLS-LDA-based two-step binary classification scheme is conducted. The first fifteen PLS scores are extracted from the SERS measurements and then analyzed by two-sample t-test to achieve the PLS scores with significant difference ($p<0.05$) between healthy and neuropsychiatric disorders, as well as between LDD and MCI patients, and then these PLS scores are used as the input of classification models. In the first step, only the 1st ($p = 0.0000$), 2nd ($p = 0.0000$), 4th ($p = 0.0095$), 6th ($p = 0.0003$), 8th ($p = 0.0326$), 11th ($p = 0.0062$) and 14th ($p = 0.0381$) PLS scores serve as the input of the classification model to differentiate healthy versus neuropsychiatric disorders. Similarly, in the second step, only the 1st ($p = 0.0011$), 2nd ($p = 0.0000$), 3rd ($p = 0.0265$) and 5th ($p = 0.0245$) PLS scores serve as the

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input of the classification model to differentiate LDD versus MCI patients. Besides, Fig. 6(a) and (b) show the corresponding loadings of the above PLS scores in the two binary classification models. It can be observed that the peaks in those PLS loadings are consistent with those SERS peaks with statistically significant differences among different groups as shown in Fig. 4(c) and Fig. 5(c), especially for the first several PLS loadings, which indicate that the PLS loadings catch the most identifying information of SERS spectra among each group. Besides, the linear discriminant scores are derived from the PLS-LDA models in the two-step binary classification scheme and plotted in Fig. 7(a) and (b), in which excellent separation between healthy control and neuropsychiatric disorders group as well as LDD group and MCI group can be clearly observed.

The performances of above models in the PLS-LDA-based two-step binary classification scheme are evaluated by LOO cross-validation with the criteria of classification accuracy, sensitivity, specificity, ROC curves and the AUC value. More specifically, the neuropsychiatric disorders group and the MCI group are treated as positive in the first and second binary classification, respectively. The total classification results of healthy, LDD and MCI groups are shown in Table 2. Specifically, in the first binary classification, the accuracy, sensitivity and specificity are 91.6%, 88.6% and 97.5%, respectively; in the second binary classification, the accuracy, sensitivity and specificity are 85.7%, 84.8% and 86.5%, respectively. Hence, the total accuracy is 83.2% for distinguishing healthy, LDD and MCI groups. Besides, the ROC curves and large AUC values in Fig. 8 can be used as further evidence to the excellent performance of our proposed method.

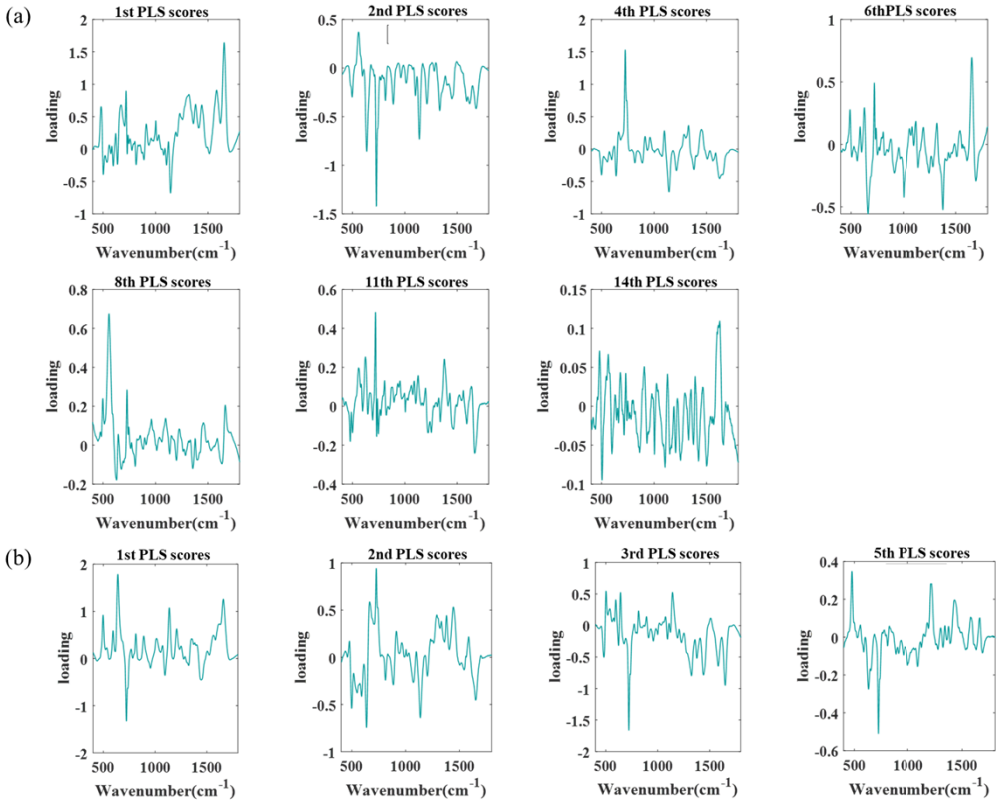

Fig. 6. The corresponding loadings of PLS scores with statistically significant differences for differentiating **(a)** the healthy and neuropsychiatric disorders groups; **(b)** the LDD and MCI groups.

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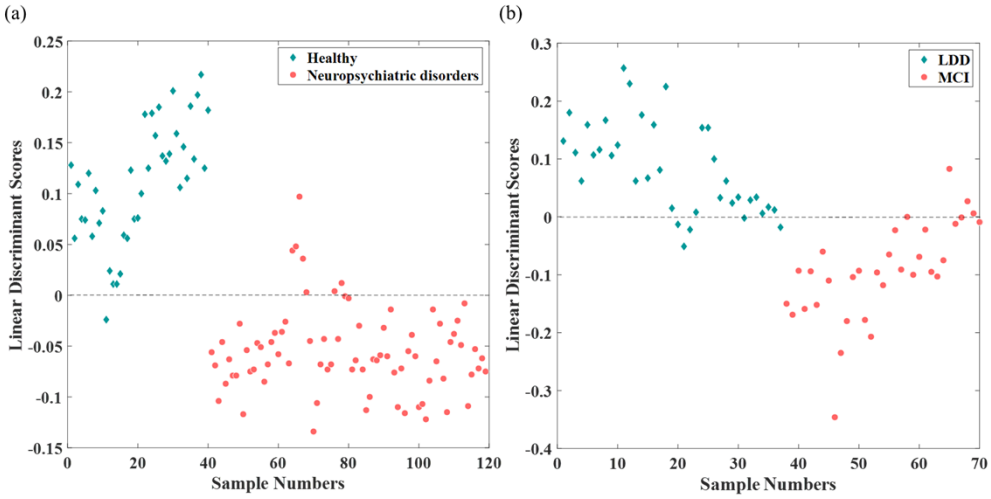

Fig. 7. Scatter plots of the linear discriminant scores for differentiating **(a)** the healthy and neuropsychiatric disorders groups; **(b)** the LDD and MCI groups.

Table 2. The overall classification results of the healthy, LDD and MCI groups by PLS-LDA-based two-step binary classification scheme with LOO cross-validation.

PLS-LDA-based two-step binary classification scheme	Sensitivity	Specificity	Accuracy
(The first binary classification) Healthy v.s. neuropsychiatric disorders	88.6% (70/79)	97.5% (39/40)	91.6% (109/119)
(The second binary classification) LDD v.s. MCI	84.8% (28/33)	86.5% (32/37)	85.7% (60/70)

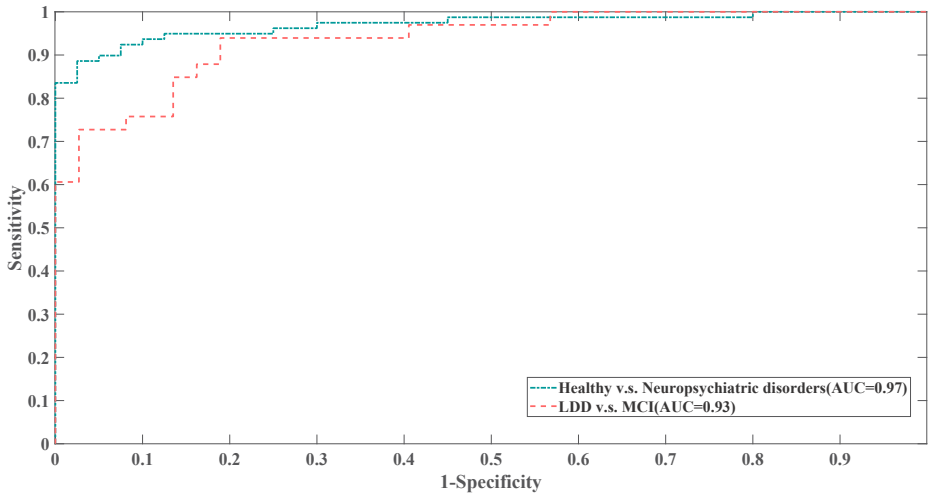

Fig. 8. The ROC curves obtained by using PLS-LDA-based two-step binary classification scheme between the healthy and neuropsychiatric disorders groups, and between the LDD and MCI groups.

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Thus, it is convincingly demonstrated that serum SERS technique combined with PLS-LDA has promising potential for accurately identifying healthy, LDD and MCI patients.

4. Conclusions

In this study, the possibility of identifying LDD and MCI patients by serum SERS technology is investigated. By comparing the average serum SERS spectra, obvious biochemical changes including ascorbic acid, saccharide, cell-free DNA and amino acids are found among different groups, which might be related to the oxidative stress, nutritional status, lipid peroxidation and metabolic abnormalities in brain. Besides, the excellent performances of the PLS-LDA-based two-step binary classification scheme furtherly indicated that the serum SERS technique can be used as a promising tool for fast, sensitive and non-invasive detection of LDD and MCI; which may bring a new way to assist in early diagnosis and timely intervention of neuropsychiatric disorders.

Funding. Natural Science Foundation of Zhejiang Province (LQ23H180004); Department of Education of Zhejiang Province (Y202146723); High-end Foreign Experts Recruitment Plan of China (GXL20200218001); Ningbo City Public welfare Science and technology Plan project (2022S025); K. C. Wong Magna Fund in Ningbo University.

Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

References

1. B. D. Lebowitz, J. L. Pearson, L. S. Schneider, C. F. Reynolds, G. S. Alexopoulos, M. L. Bruce, Y. Conwell, I. R. Katz, B. S. Meyers, M. F. Morrison, J. Mossey, G. Niederehe, and P. Parmelee, "Diagnosis and Treatment of Depression in Late Life: Consensus Statement Update," JAMA **278**(14), 1186–1190 (1997).
2. R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Merikangas, and E. E. Walters, "Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication," Arch. Gen. Psychiatry **62**(6), 593–602 (2005).
3. A. P. Porsteinsson, R. S. Isaacson, S. Knox, M. N. Sabbagh, and I. Rubino, "Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021," J Prev Alz Dis **8**(3), 1–16 (2021).
4. D. Y. Fan and Y. J. Wang, "Early Intervention in Alzheimer's Disease: How Early is Early Enough?" Neurosci. Bull. **36**(2), 195–197 (2020).
5. F. L. Guest, "Early Detection and Treatment of Patients with Alzheimer's Disease: Future Perspectives," Adv. Exp. Med. Biol. **1118**, 295–317 (2019).
6. Q. F. Zhao, L. Tan, H. F. Wang, T. Jiang, M. S. Tan, L. Tan, W. Xu, J. Q. Li, J. Wang, T. J. Lai, and J. T. Yu, "The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis," J. Affective Disord. **206**, 8 (2016).
7. B. S. Diniz, M. A. Butters, S. M. Albert, M. A. Dew, and C. F. Reynolds, "Late-life depression and risk of vascular dementia and Alzheimer's disease: systematic review and meta-analysis of community-based cohort studies," Br. J. Psychiatry **202**(5), 329–335 (2013).
8. V. Kiyasova, M. Caillaud, E. Bouguen, I. Guignot, H. Schneble, F. Bonneville, F. Lala, N. Sastre, P.J. Ousset, M. Soto, J. Delrieu, M. Pueyo, and B. Vellas, "Longitudinal evaluation of clinical symptoms in controls, MCI, and Alzheimer's disease patients from the rosas study," Alzheimer's Dementia **11**(7S_Part_8), 379–380 (2015).
9. W. M. van Oostveen and E. C. M. de Lange, "Imaging Techniques in Alzheimer's Disease: A Review of Applications in Early Diagnosis and Longitudinal Monitoring," Int. J. Mol. Sci. **22**(4), 2110 (2021).
10. M. S. Henry, A. P. Passmore, S. Todd, B. McGuinness, D. Craig, and J. A. Johnston, "The development of effective biomarkers for Alzheimer's disease: a review," Int J Geriatr Psychiatry **28**(4), 331–340 (2013).
11. B. Olsson, R. Lautner, U. Andreasson, A. Öhrfelt, E. Portelius, M. Bjerke, M. Hölttä, C. Rosén, C. Olsson, G. Strobel, E. Wu, K. Dakin, M. Petzold, K. Blennow, and H. Zetterberg, "CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis," Lancet Neurol. **15**(7), 673–684 (2016).
12. A. Misrani, S. Tabassum, and L. Yang, "Mitochondrial Dysfunction and Oxidative Stress in Alzheimer's Disease," Front. Aging Neurosci. **13**, 617588 (2021).
13. F. Panza, V. Frisardi, D. Seripa, B. P. Imbimbo, D. Sancarlo, G. D'Onofrio, F. Addante, F. Paris, A. Pilotto, and V. Solfrizzi, "Metabolic syndrome, mild cognitive impairment, and dementia," Curr. Alzheimer Res. **8**(5), 492–509 (2011).
14. R. Marijnissen, N. Vogelzangs, M. Mulder, R. van den Brink, H. Comijs, and R. Oude Voshaar, "Metabolic dysregulation as predictor for the course of late-life depression," Eur. psychiatr. **33**(S1), S416 (2016).

Biomedical Optics EXPRESS

15. D. Andrew and E. Alistair, "Raman spectroscopy and related techniques in Biomedicine," Sensors **10**(3), 1871–1889 (2010).
16. O. A. Magsumova, V. A. Polkanova, E. V. Timchenko, and L. T. Volova, "Raman spectroscopy and its application in different areas of medicine," Stomat. **100**(4), 137–142 (2021).
17. D. Cialla, A. März, R. Böhme, F. Theil, K. Weber, M. Schmitt, and J. Popp, "Surface-enhanced Raman spectroscopy (SERS): progress and trends," Anal. Bioanal. Chem. **403**(1), 27–54 (2012).
18. E. C. Le Ru, E. Blackie, M. Meyer, and P. G. Etchegoin, "Surface Enhanced Raman Scattering Enhancement Factors: A Comprehensive Study," J. Phys. Chem. C **111**(37), 13794–13803 (2007).
19. A. G. Carota, B. Campanella, R. Del Carratore, P. Bongioanni, R. Giannelli, and S. Legnaioli, "Raman spectroscopy and multivariate analysis as potential tool to follow Alzheimer's disease progression," Anal. Bioanal. Chem. **414**(16), 4667–4675 (2022).
20. M. Paraskevaidi, C. L. M. Morais, D. E. Halliwell, D. M. A. Mann, D. Allsop, P. L. Martin-Hirsch, and F. L. Martin, "Raman Spectroscopy to Diagnose Alzheimer's Disease and Dementia with Lewy Bodies in Blood," ACS Chem. Neurosci. **9**(11), 2786–2794 (2018).
21. K. Wang, D. W. Sun, H. Pu, and Q. Wei, "Shell thickness-dependent Au@Ag nanoparticles aggregates for high-performance SERS applications," Talanta **195**, 506–515 (2019).
22. G. Frens, "Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions," Nature (London), Phys. Sci. **241**(105), 20–22 (1973).
23. S. Chen, S. Zhu, X. Cui, W. Xu, C. Kong, Z. Zhang, and W. Qian, "Identifying non-muscle-invasive and muscle-invasive bladder cancer based on blood serum surface-enhanced Raman spectroscopy," Biomed. Opt. Express **10**(7), 3533–3544 (2019).
24. S. Chen, H. Lin, H. Zhang, F. Guo, S. Zhu, X. Cui, and Z. Zhang, "Identifying functioning and nonfunctioning adrenal tumors based on blood serum surface-enhanced Raman spectroscopy," Anal. Bioanal. Chem. **413**(16), 4289–4299 (2021).
25. A. Bonifacio, S. Dalla Marta, R. Spizzo, S. Cervo, A. Steffan, A. Colombatti, and V. Sergo, "Surface-enhanced Raman spectroscopy of blood plasma and serum using Ag and Au nanoparticles: a systematic study," Anal. Bioanal. Chem. **406**(9-10), 2355–2365 (2014).
26. J.L. Koenig, "Raman spectroscopy of biological molecules: A review," J. Polym. Sci. Macromol. Rev. **6**(1), 59–177 (1972).
27. J. De Gelder, K. De Gussem, P. Vandenabeele, and L. Moens, "Reference database of Raman spectra of biological molecules," J. Raman Spectrosc. **38**(9), 1133–1147 (2007).
28. J. S. Metcalf, R. A. Dunlop, J. T. Powell, S. A. Banack, and P. A. Cox, "L-Serine: a Naturally-Occurring Amino Acid with Therapeutic Potential," Neurotoxic. Res. **33**(1), 213–221 (2018).
29. K. Iqbal, F. Liu, and C. X. Gong, "Tau and neurodegenerative disease: the story so far," Nat. Rev. Neurol. **12**(1), 15–27 (2016).
30. L. Buée, T. Bussière, V. Buée-Scherrer, A. Delacourte, and P. R. Hof, "Tau protein isoforms, phosphorylation and role in neurodegerative disorders," Brain Res. Rev. **33**(1), 95–130 (2000).
31. J. W. Gawryluk, J. F. Wang, A. C. Andreazza, L. Shao, and L. T. Young, "Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders," Int. J. Neuropsychopharmacol. **14**(01), 123–130 (2011).
32. F. Gu, V. Chauhan, and A. Chauhan, "Glutathione redox imbalance in brain disorders," Curr. Opin. Clin. Nutr. Metab. Care **18**(1), 89–95 (2015).
33. K. Owens, J. H. Park, R. Schuh, and T. Kristian, "Mitochondrial Dysfunction and NAD+ Metabolism Alterations in the Pathophysiology of Acute Brain Injury," Transl. Stroke Res. **4**(6), 618–634 (2013).
34. L. G. Nucifora, T. Tanaka, L. N. Hayes, M. Kim, B. J. Lee, T. Matsuda, F. C. Nucifora, T. Sedlak Jr, R. Mojtabai, W. Eaton, and A. Sawa, "Reduction of plasma glutathione in psychosis associated with schizophrenia and bipolar disorder in translational psychiatry," Transl. Psychiatry **7**(8), e1215 (2017).
35. T. Best, E. Kemps, and J. Bryan, "Effects of Saccharides on Brain Function and Cognitive Performance," Nutr. Rev. **63**(12), 409–418 (2005).
36. L.S. Nidadavolu, D.M. Feger, A.L. Gross, D.A. Bennett, J. Walston, P. Abadir, and E.S. Oh, "Circulating cell-free DNA of mitochondrial origin connects cognitive and physical decline in aging and is associated with increased mortality," Alzheimer's Dementia **16**(S2), e045595 (2020).
37. E. Socha, P. Kośliński, M. Koba, K. Mądra-Gackowska, K. Kędziora-Kornatowska, M. Gackowski, and E. Daghir-Wojtkowiak, "Amino Acid Levels as Potential Biomarker of Elderly Patients with Dementia," Brain Sci. **10**(12), 914 (2020).
38. G. Corso, A. Cristofano, N. Sapere, G. la Marca, A. Angiolillo, M. Vitale, R. Fratangelo, T. Lombardi, C. Porcile, M. Intrieri, and A. Di Costanzo, "Serum Amino Acid Profiles in Normal Subjects and in Patients with or at Risk of Alzheimer Dementia," Dement. Geriatr. Cogn. Dis. Extra **7**(1), 143–159 (2017).
39. A. N. Fonteh, R. J. Harrington, A. Tsai, P. Liao, and M. G. Harrington, "Free amino acid and dipeptide changes in the body fluids from Alzheimer's disease subjects," Amino Acids **32**(2), 213–224 (2007).
40. Z. Chen and C. Zhong, "Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: Implications for diagnostic and therapeutic strategies," Prog. Neurobiol. **108**, 21–43 (2013).

Biomedical Optics EXPRESS

41. S. Gariballa, "Poor Vitamin C Status is Associated with Increased Depression Symptoms Following Acute Illness in Older People," Int. J. Vitam. Nutr. Res. **84**(1-2), 12–17 (2014).
42. D. Plevin and C. Galletly, "The neuropsychiatric effects of vitamin C deficiency: a systematic review," BMC Psychiatry **20**(1), 315 (2020).
43. F.E. Harrison, "A Critical Review of Vitamin C for the Prevention of Age-Related Cognitive Decline and Alzheimer's Disease," J. Alzheimer's Dis. **29**(4), 711–726 (2012).
44. N. Travica, K. Ried, A. Sali, I. Hudson, A. Scholey, and A. Pipingas, "Plasma Vitamin C Concentrations and Cognitive Function: A Cross-Sectional Study," Front. Aging Neurosci. **11**, 72 (2019).
45. S. Dixit, A. Bernardo, J. M. Walker, J. A. Kennard, G. Y. Kim, E. S. Kessler, and F. E. Harrison, "Vitamin C deficiency in the brain impairs cognition, increases amyloid accumulation and deposition, and oxidative stress in APP/PSEN1 and normally aging mice," ACS Chem. Neurosci. **6**(4), 570–581 (2015).
46. N. Schupf, M. X. Tang, H. Fukuyama, J. Manly, H. Andrews, P. Mehta, J. Ravetch, and R. Mayeux, "Peripheral A beta subspecies as risk biomarkers of Alzheimer's disease," Proc. Natl. Acad. Sci. U. S. A. **105**(37), 14052–14057 (2008).
47. J. A. Hardy and G. A. Higgins, "Alzheimer's disease: The amyloid cascade hypothesis," Science **256**(5054), 184–185 (1992).
48. P. S. Insel, M. C. Donohue, R. S. Mackin, P. S. Aisen, O. Hansson, M. W. Weiner, and N. Mattsson, "Cognitive and functional changes associated with Aβ pathology and the progression to mild cognitive impairment," Neurobiol. Aging **48**, 172–181 (2016).
49. P.T. Martin, "Glycobiology of the synapse," Glycobiology **12**(1), 1R–7 (2002).
50. J. Arslan, H. Jamshed, and H. Qureshi, "Early Detection and Prevention of Alzheimer's Disease: Role of Oxidative Markers and Natural Antioxidants," Front. Aging Neurosci. **12**, 231 (2020).
51. G. Morris, K. R. Walder, M. Berk, W. Marx, A. J. Walker, M. Maes, and B. K. Puri, "The interplay between oxidative stress and bioenergetic failure in neuropsychiatric illnesses: can we explain it and can we treat it?" Mol. Biol. Rep. **47**(7), 5587–5620 (2020).